Review

Metabolism and degradation of glyphosate in aquatic cyanobacteria: A review

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Use of glyphosate (N-phosphonomethylglycine), a broad-spectrum, non-selective, post-emergence herbicide has been increased steadily with the introduction of genetically modified glyphosate-resistant crops. Increased reliance on herbicides for suppressing weeds and aggressive marketing have also contributed substantially to rising demand for glyphosate. Degradation of glyphosate was basically done by soil microorganisms; however, once the herbicide reached to the aquatic systems, cyanobacterial strains were reported to be involved in the process of biodegradation. Upon glyphosate exposure, a remarkable tolerance was reported in many strains, where cell proliferation was found to be completely unaffected by the herbicide at the concentration of micromolar to millimolar range. However, the mechanism through which cyanobacteria exhibit the tolerance seemed to be widely varied and species-dependent. Carrier-independent uptake of glyphosate has been suggested as the resistance mechanism at micromolar level concentrations. Presence of resistant form of the target enzyme EPSP (5-enolpyruvylshikimate-3-phosphate) and the ability of some strains to metabolize glyphosate have also been reported to be responsible for the tolerance. A remarkable ability to degrade glyphosate has been identified from some cyanobacterial strains such as Spirulina spp. where degradative pathway was however reported to be different from those exhibited in other bacteria. Exploitation of cyanobacteria in biological treatments of waste water contaminated with glyphosate has not yet been reported, mainly due to lack of research evidence on as to how cyanobacteria deal with biodegradation of glyphosate under field conditions.

Key words: Glyphosate, cyanobacteria, biodegradation, biological treatments.

GLYPHOSATE USAGE AND MODE OF ACTION

Glyphosate (N-phosphonomethylglycine), a broad-spectrum, non-selective, post-emergence herbicide is widely used in suppressing annual and perennial weeds in agricultural lands, ornamental and residential gardens and in aquatic systems (Walpola et al., 2007; Lipok et al., 2010). The herbicide is also used in silviculture for controlling undesirable competing vegetation that may emerge after harvesting of high-yield coniferous plantations. Some glyphosate based herbicides specifically formulated to be used as aquatic herbicides are employed extensively to control noxious aquatic weeds and algal blooms (Siemering et al., 2008). Due to its widespread usage over the years, glyphosate is now considered to be the most studied organophosphonate (Lipok et al., 2009). Though, different chemical formulations are commercially available, glyphosate is generally formulated in its form.

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of isopropylamine salt (IPA salt) (Figure 1). However, commercial preparations (for example, Roundup®) were reported to be more toxic than glyphosate alone (Tsui and Chu, 2003; Cedergreen and Streibig, 2005; Sobrojo et al., 2007). Agricultural use of glyphosate greatly expanded with the development of minimum and no-till cultivation systems, where application of herbicides prior to planting became a standard practice (Walpola et al., 2007). No-till practice is found to be rapidly adopted around the world (Altiere and Pengue, 2006) as it improves soil quality avoiding organic matter lost (Bayer et al., 2006) and water evaporation and protects soil from erosion (Bollinger et al., 2006). Furthermore, this practice is economically affordable for a wide range of farming communities. Use of glyphosate further expanded with the introduction of genetically modified glyphosate-resistant crops (Woodburn, 2000), which mainly include soybean, maize, cotton, canola and sugar beet (Duke, 2011). However, as time progressed, less sensitive and herbicide-resistant weed species are reported to be evolved (Pérez and Kogan, 2003; Powles, 2008; Binemelis et al., 2009) forcing farmers to increase the average rate of glyphosate application per unit area. The global increase in usage of glyphosate is also associated with aggressive marketing, as well as with the increased reliance on herbicides for controlling weeds (Pengue, 2005). Glyphosate does possess slow mode of action, which ensures distribution of the herbicide throughout the plant before appearing the symptoms. In fact, once enter to plants, metabolic degradation of glyphosate takes place slowly or sometimes no degradation at all, thus highly effective for many plant species. As reported by Cerdeira and Duke (2006), the shikimate pathway is blocked by glyphosate through inhibition of 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). Consequently, protein synthesis is halted due to deregulation of the shikimate pathway and inhibition of the formation of aromatic amino acids tryptophan, tyrosine and phenylalanine (Duke et al., 2003). As symptoms, growth is inhibited immediately after application, followed by foliar chlorosis and necrosis is shown within 4 to 7 days in highly susceptible species (Sensenman, 2007). However, it may take 2 to 3 weeks to display symptoms in susceptible species.

As a post emergence herbicide, glyphosate is recommended at doses ranging 0.21 and 4.2 kg a.i. per ha depending on the use (Vencill, 2002). In most of the agricultural lands, the application dose is generally over 1 kg per ha (Cerdeira and Duke, 2006).

SOIL EXPOSURE AND CONTAMINATION OF AQUATIC SYSTEMS

Upon exposure, glyphosate is usually assumed to be tightly adsorbed to soil, though its persistence in soil depends on the climate and soil characteristics (Perez et al., 2007). However, Helander et al. (2012) reported that glyphosate is neither entirely no immediately degraded in soils, where degradation is mainly done by soil microorganisms. Despite glyphosate is found to be toxic to several microbes (Busse et al., 2001), growth enhancement is also reported from some microbes (Kremer and Means, 2009). Thus, the presence of glyphosate can result in alterations in soil microbial community structure (Lancaster et al., 2010). As stated by Helander et al. (2012), the effects of glyphosate and its main degradation product aminomethylphosphonic acid (AMPA) on soil microorganisms and biological interactions can be complex and multidirectional. As of the published literature, the average half-life of glyphosate in soil varies between 2 and 197 days (WHO, 1988; Giesey et al., 2000); thus, a typical field half-life of 47 days has been suggested (Vencill, 2002). Due to the mobility of glyphosate in soil, contamination of groundwater could also be possible through leaching. However, such leaching is reported to be restricted to some special climatic, soil and spraying conditions (Vereecken, 2005; Borggaard and Gimsing, 2008; Laitinen, 2006). In this context, a considerable rate of glyphosate leaching has been reported in very coarse (gravelly) materials with limited retention capacity (Torstensson et al., 2005). As reported by Kjaer et al. (2005) and Candela et al. (2010), excessive irrigation or rainfall immediately after application could also be responsible for increasing risk of leaching. Though not significant, terrestrial applications of glyphosate can also result in contamination of aquatic systems through accidental offsite movement in herbicide spray drift, or through surface run-off (Abrate et al., 2009). Unacceptable habits such as washing and cleaning the tanks of the fumigation machines in streams and adjacent water bodies near cultivation fields may also result in reaching glyphosate to the water bodies.

In waters, glyphosate is found to be chemically stable because it does not undergo photochemical degradation (Lipok et al., 2010). As stated by Scribner et al. (2007), glyphosate and AMPA are among the frequently reported pesticides detected in water-pollution monitoring. While degradation, AMPA is formed by other phosphonate compounds also (Kolpin et al., 2006; Botta et al., 2009), thus glyphosate could not be considered as the main
source of AMPA (Trass and Smit, 2003). Pérez et al. (2007) elaborating the possible impacts of glyphosate on the structure of the phytoplankton and periphyton communities in fresh water, reduction in total micro- and nanophytoplankton has been reported. Glyphosate could have a direct effect on the periphytic colonization of substrata (Vera et al., 2010). Therefore, it is obvious that non-target periphyton and phytoplankton communities in aquatic ecosystems could be affected by the toxicity of glyphosate. The application of glyphosate in fresh water systems may result in shifting the community from glyphosate-sensitive green algae and diatoms to glyphosate-tolerant cyanobacteria (Saxton et al., 2011). Indirect effects via the eutrophication potential of glyphosate degradation should also be taken into account; thus, overall functioning of the aquatic ecosystems and the basis of food webs may be potentially affected by glyphosate.

Alterations in microbial community structure upon glyphosate exposure have been described in marine environments also (Stachowski-Haberkorn et al., 2008). In water, the average half-life of glyphosate may vary from a few days to 91 days (Tomlin, 2006).

**EFFECT OF GLYPHOSATE ON CYANOBACTERIA**

Cyanobacteria, a highly diverse group of prokaryotic microorganisms exhibiting oxygenic photosynthesis has gained high recognition as the most efficient solar energy harvesters among all the living organisms (Kulasooiriya, 2011). They could be found in all most all kind of habitats including extreme terrestrial and aquatic environments, thus account for a major proportion of the total phytoplankton biomass (Arunkumara, 2012). Due to their remarkable ecophysiological adaptation, they are able to cope with different kind of stress conditions (Lee et al., 2003; Barton et al., 2004; Dyhrman et al., 2006; Panikh et al., 2006). However, they have not yet been fully exploited for biological treatment of polluted waters, and only scanty information is available on as to how cyanobacteria participates in the process of biodegradation of chemical pollutants. Six cyanobacterial strains (Anabaena spp., Arthrospira fusiformis, Leptolyngbya boryana, Microcystis aeruginosa, Nostoc punctiforme, Spirulina platensis) were employed in a laboratory investigation to examine the basis of their resistance to glyphosate (Forlani et al., 2008). Remarkable tolerance was observed in all the strains, where cell proliferation was found to be completely unaffected by the herbicide in the micromolar to millimolar range. Quite interestingly, A. fusiformis and S. platensis have not showed any significant increase in their doubling time even at the highest dose tested (10 mM). Lipok et al. (2007) conducted a laboratory investigation with a mixed culture of Spirulina spp. and reported that the growth was not affected with the addition of glyphosate at low concentration (0.2 mM). They have observed a short growth cycle (maximal cell density at 5 to 6 days after the inoculation). However, as concentration increased over 20 mM, a significant reduction in growth was observed in a dose-dependent manner.

Explaining their results with *M. aeruginosa*, López-Rodas et al. (2007) reported that rare spontaneous preselective mutations could assist in surviving the strain against glyphosate stress. Furthermore, they quoted that such mutants can be expected only if appropriate genetic variability is available within the species. Their comment is in line with Whitton (2002), who reported formation of a glyphosate resistance population of *M. aeruginosa* from cells with slightly different morphology. Whole-cell system with *S. platensis* cells grown in standard medium was employed in elucidating the ability of cyanobacteria to metabolize glyphosate (Forlani et al., 2008). Based on the findings, they stated that the possible act of carrier-independent uptake of glyphosate at micromolar level concentrations which results in remarkable tolerance to the herbicide. Therefore, at micromolar level concentrations, cell impermeability attributed largely to the tolerance mechanism exhibited in *S. platensis*. According to them, the initial tolerance up to 20 mM is in line with the previous reports (Sikha and Singh, 2004; Singh and Datta, 2005) on varying resistance of cyanobacteria to this herbicide.

Powell et al. (1992) reported that tolerance of *Anabaena variabilis* ATCC 29413 is related to the presence of a resistant form of the target enzyme EPSP, which is consistent with the findings of Forlani et al. (2008) for *Anabaena* spp and *N. punctiforme*. Based on this, it is quite reasonable to consider that the presence of an insensitive variant of the target enzyme as a biochemical basis of *in vivo* tolerance. However, they have observed a significant growth inhibition at levels at which enzyme activity was completely unaffected, thus effect of *in vivo* may be higher than that of *in vitro*. Having considered all these reports, Forlani et al. (2008) suggested that tolerance is attributed to several mechanisms work in a cooperative manner. Making similar remarks, Vera et al. (2010) reported that cyanobacteria may resist glyphosate by different strategies. Among them, the overproduction of EPSP synthase or the production of a glyphosate-tolerant enzyme (Powell et al., 1991) and degradation of glyphosate and use it as a phosphorus source (Forlani et al., 2008) are still considered to be dominant, despite the variations reported among different species. However, contrary to this, Powell et al. (1991) have reported that no evidence of glyphosate degradation could be observed with *Synechocystis* PCC 6803 and *A. variabilis* ATCC 29413 though the species exhibited a high degree of tolerance to glyphosate. According to them, toxicity differed with the type of formulations (Roundup > isopropylamine salt > free acid) and correlated with their rates of uptake.

Taking all aforementioned into account, it is quite reasonable to state that aquatic systems may not frequently
**Table 1.** The effect of glyphosate on several cyanobacterial strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Concentration</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Synechocystis</em> Sauvageau (PCC 6803)</td>
<td>0.5 - 20 mM</td>
<td>Confirmed tolerance even at 20 mM</td>
<td>Powell et al. (1991)</td>
</tr>
<tr>
<td><em>Anabaena variabilis</em> Kutz (ATCC 29413)</td>
<td></td>
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<tr>
<td><em>S. (Arthrospira) platensis</em></td>
<td></td>
<td>Confirmed tolerance</td>
<td>Lipok et al. (2010)</td>
</tr>
<tr>
<td><em>Arthrospira fusiformis</em></td>
<td></td>
<td>Confirmed tolerance</td>
<td></td>
</tr>
<tr>
<td><em>Nostoc punctiforme</em></td>
<td></td>
<td>Confirmed tolerance</td>
<td></td>
</tr>
<tr>
<td><em>Anabaena catenula</em></td>
<td>0.07 mM</td>
<td>Sensitive</td>
<td></td>
</tr>
<tr>
<td><em>Synechocystis aquatilis</em></td>
<td></td>
<td>Confirmed tolerance</td>
<td></td>
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<tr>
<td><em>Microcystis aeruginosa</em></td>
<td></td>
<td>Confirmed tolerance</td>
<td></td>
</tr>
<tr>
<td><em>Leptolyngbya boryana</em></td>
<td></td>
<td>Confirmed tolerance up to 20 mM, thereafter</td>
<td>Lipok et al. (2007)</td>
</tr>
<tr>
<td><em>Spirulina</em> spp</td>
<td>0.2 - 20 mM</td>
<td>Confirmed tolerance in the micromolar to millimolar range</td>
<td>Forlani et al. (2008)</td>
</tr>
<tr>
<td><em>Anabaena</em> sp. ATCC 27347 (PCC 7120)</td>
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<tr>
<td><em>Arthrospira fusiformis</em> CCALA 023</td>
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<tr>
<td><em>Leptolyngbya boryana</em> ATCC 27894 (PCC 6306)</td>
<td>0.01 - 10mM</td>
<td>Confirmed tolerance in the micromolar to millimolar range</td>
<td>Forlani et al. (2008)</td>
</tr>
<tr>
<td><em>Microcystis aeruginosa</em> PCC 7941 (CCALA 106)</td>
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<tr>
<td><em>Nostoc punctiforme</em> ATCC 29133 (PCC 73102)</td>
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<tr>
<td><em>Spirulina platensis</em> C1 (PCC 9438)</td>
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<tr>
<td><em>Microcystis aeruginosa</em></td>
<td>0 - 110 ppm</td>
<td>Sensitive, growth was totally inhibited at 110 ppm (0.7 mM)</td>
<td>Lo´pez-Rodas et al. (2007)</td>
</tr>
</tbody>
</table>

receive glyphosate at a concentration enough for suppressing cyanobacterial growth (Forlani et al., 2008), because, though applied at a recommended dose ranging 0.21 and 4.2 kg a.i. per ha depending on the use (Vencill, 2002), substantial amount of off-targeted glyphosate is adsorbed to soil particles, minimizing significant leaching or removal by means of surface run-off, which might ultimately reach to neighbouring waters. Table 1 summarizes the effect of glyphosate on several cyanobacterial strains.

**DEGRADATION OF GLYPHOSATE**

The presence of chemically and thermally stable C-P bond, a characteristic feature of glyphosate (Singh and Walker, 2006) is a matter of frequent concern, because, the C-P linkage is found to be heavily resistant to non-biological degradation in the environment (Hayes et al., 2000). As per the published literature, biodegradation of glyphosate is believed to be done basically by soil microorganisms and the process can be described under two different metabolic pathways (Duke, 2011). One process involves in splitting the glyphosate C-N bond by the action of glyphosate oxidoreductase (GOX) enzyme to produce aminomethylphosphonic acid (AMPA) and glyoxylate (Schuette, 1998). In fact, glyoxylate is not only a metabolite derived of glyphosate degradation, but also a plant endogenous metabolite involved in different metabolic pathways (Rojano-Delgado et al., 2010). By the action of the enzyme C-P lyase, AMPA, the other main metabolite is degraded to methylamine, which ultimately generates formaldehyde by the action of methylamine dehydrogenase enzyme (Lerbs et al., 1990). Formaldehyde quickly reacts with water and/or hydroxyl radicals to form methanol. Thus, the ultimate yield of the glyphosate degradation may contain carbon dioxide, phosphate, ammonia and methanol (Araujo et al., 2003). In the second pathway, sarcosine (N-methyl-glycine) is yielded through direct degradation of gly-phosate by the action of C-P lyase enzyme (Dick and Quinn, 1995; Schuette, 1998; Kafarski et al., 2000). The sarcosine can further be degraded into amino acids such as glycine, serine, cysteine, methionine and histidine (Pipke et al., 1987). Based on the aforementioned, Rojano-Delgado et
al. (2010) stated that the availability of these metabolites and their relative percentage can be used in assessing glyphosate metabolism in plants. In addition to the two common pathways aforementioned, Pizzul et al. (2009) reported that liginolytic enzymes of soil microflora do have the ability to cleave the C-P bond of glyphosate. In the presence of manganese oxide, cleavage of C-P bond of glyphosate could also be witnessed non-enzymatically though it has not often been reported in soil (Barrett and McBride, 2005). A large number of soil microorganisms such as bacteria, fungi, actinomycetes and some unidentified microbes are reported to be involved in glyphosate degradation (Borggaard and Gimsing, 2008). Furthermore, as reported by Gimsing et al. (2004) for *Pseudomonas* spp., the degradation rate is strongly correlated with the population size of soil microbes.

With regard to degradation in aqueous mediums, Lipok et al. (2007) concluding their findings with mixed culture of *Spirulina* spp, reported that the species exhibited a remarkable ability to degrade glyphosate, where the rate of glyphosate disappearance from the medium was independent of its initial concentration. They suggested that the degradative pathway for glyphosate in *Spirulina* spp. might differ from those exhibited in other bacteria. According to them, occurrence of herbicide metabolism in *Spirulina* is evident, because, the species can grow in a medium containing phosphonate as the only source of phosphorus, where the rate of herbicide transformation was found to be dependent upon the cells’ phosphorus status. In fact, Lipok et al. (2009) re-confirmed the ability of the cyanobacterium *S. platensis* and bacterium *Streptomyces lusitanus* to catalyze glyphosphate metabolism. According to Forlani et al. (2008), four cyanobacterial strains (*Anabaena* sp., *L. boryana*, *M. aeruginosa* and *N. punctiforme*) out of the six strains studied were able to use the glyphosate as the sole source of phosphorus. Dyhrman et al. (2006) too stated that the existence of phosphorous-dependent glyphosphate transformation with marine cyanobacterium *Trichodesmium erythraeum*. Glyphosate as a source of nitrogen for microorganism was also reported (Klimek et al., 2001) with *Penicillium chrysogenum* and then with *Alternaria alternate* (Lipok et al., 2003). However, reports on the utilization of glyphosate as a source of nitrogen by cyanobacteria are not yet available in the literature. Elaborating their findings with cyanobacterium *A. variabilis*, Ravi and Balakumar (1998) reported that extracellular phosphatases are able to hydrolyze the C-P bond of glyphosate; however, this claim has not been reiterated so far by the other authors.

Forlani et al. (2008) based on their results, stated that extracellular phosphatases seems unlikely to contribute any substantial scale to glyphosate degradation. As described earlier, different steps are reported to be involved in the process of glyphosate degradation in different strains of microorganisms. Some of them in fact can utilize glyphosate as a source of nutrient. In this regard, cyanobacterial strains which possess the ability to use this phosphonate as a source of phosphorus is of practically significance, because, such strains could effectively be employed in treating the problematic waters.

**CONCLUSION**

It is quite obvious that the use of glyphosate has brought impressive economical benefits, in particular, through the enhanced agricultural productivity. However, due to high percentage of applied glyphosate is often reported to be deposited on non-target areas, contamination of soil and water is inevitable. Under this background, frequent assessments on the impacts to non-target organisms are of prime importance. However, the vast majority of the present investigations dealing with the impact of glyphosate on aquatic organisms and mechanism involved in degradation are based on laboratory bioassays. Furthermore, toxicity studies are often targeted on individual strains, because, such findings assist in assessing the direct impacts of herbicide on the organisms of concern. However, generation of remediation strategies for contaminated waters, merely based on such findings would not be advisable. Thus, field studies conducted in natural environments are encouraged as they could come up with much broader understanding as to how cyanobacteria cope with glyphosate toxicity.

**REFERENCES**


